

CLAIMS

What is claimed is:

- 5 1. A proteorhodopsin gene, comprising an isolated DNA sequence for encoding a proteorhodopsin protein.
2. The proteorhodopsin gene of claim 1, wherein said proteorhodopsin gene is retrieved from a genomic fragment of a sample of naturally occurring bacteria.
- 10 3. The proteorhodopsin gene of claim 2, wherein said naturally occurring bacteria are marine proteobacteria.
4. The proteorhodopsin gene of claim 2, wherein said naturally occurring bacteria are SAR86 bacteria.
- 15 5. The proteorhodopsin gene of claim 2, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.
- 20 6. The proteorhodopsin gene of claim 5, wherein said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.
- 25 7. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC31A8, said proteorhodopsin gene is Sequence ID No:4 and said proteorhodopsin protein is Sequence ID No:5.

8. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC40E8, said proteorhodopsin gene is Sequence ID No:8 and said proteorhodopsin protein is Sequence ID No:9.

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9. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC41B4, said proteorhodopsin gene is Sequence ID No:10 and said proteorhodopsin protein is Sequence ID No:11.

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10. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone BAC64A5, said proteorhodopsin gene is Sequence ID No:12 and said proteorhodopsin protein is Sequence ID No:13.

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11. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT0m1, said proteorhodopsin gene is Sequence ID No:14 and said proteorhodopsin protein is Sequence ID No:15.

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12. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m1, said proteorhodopsin gene is Sequence ID No:16 and said proteorhodopsin protein is Sequence ID No:17.

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13. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m3, said proteorhodopsin gene is Sequence ID No:18 and said proteorhodopsin protein is Sequence ID No:19.

14. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m4, said proteorhodopsin gene is Sequence ID No:20 and said proteorhodopsin protein is Sequence ID No:21.

15. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone HOT75m8, said proteorhodopsin gene is Sequence ID No:22 and said proteorhodopsin protein is Sequence ID No:23.

16. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB0m1, said proteorhodopsin gene is Sequence ID No:24 and said proteorhodopsin protein is Sequence ID No:25.

17. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB0m2, said proteorhodopsin gene is Sequence ID No:26 and said proteorhodopsin protein is Sequence ID No:27.

18. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB20m2, said proteorhodopsin gene is Sequence ID No:28 and said proteorhodopsin protein is Sequence ID No:29.

19. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB20m5, said proteorhodopsin gene is Sequence ID No:30 and said proteorhodopsin protein is Sequence ID No:31.

20. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB20m12, said proteorhodopsin gene is Sequence ID No:32 and said proteorhodopsin protein is Sequence ID No:33.

5 21. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB40m1, said proteorhodopsin gene is Sequence ID No:34 and said proteorhodopsin protein is Sequence ID No:35.

10 22. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB40m5, said proteorhodopsin gene is Sequence ID No:36 and said proteorhodopsin protein is Sequence ID No:37.

15 23. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB40m12, said proteorhodopsin gene is Sequence ID No:38 and said proteorhodopsin protein is Sequence ID No:39.

20 24. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m5, said proteorhodopsin gene is Sequence ID No:40 and said proteorhodopsin protein is Sequence ID No:41.

25. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m7, said proteorhodopsin gene is Sequence ID No:42 and said proteorhodopsin protein is Sequence ID No:43.

26. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m9, said proteorhodopsin gene is Sequence ID No:44 and said proteorhodopsin protein is Sequence ID No:45.
- 5 27. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone MB100m10, said proteorhodopsin gene is Sequence ID No:46 and said proteorhodopsin protein is Sequence ID No:47.
- 10 28. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB1, said proteorhodopsin gene is Sequence ID No:48 and said proteorhodopsin protein is Sequence ID No:49.
- 15 29. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB2, said proteorhodopsin gene is Sequence ID No:50 and said proteorhodopsin protein is Sequence ID No:51.
- 20 30. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB5, said proteorhodopsin gene is Sequence ID No:52 and said proteorhodopsin protein is Sequence ID No:53.
31. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB7, said proteorhodopsin gene is Sequence ID No:54 and said proteorhodopsin protein is Sequence ID No:55.

32. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB6, said proteorhodopsin gene is Sequence ID No:56 and said proteorhodopsin protein is Sequence ID No:57.
- 5 33. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALB8, said proteorhodopsin gene is Sequence ID No:58 and said proteorhodopsin protein is Sequence ID No:59.
- 10 34. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALE1, said proteorhodopsin gene is Sequence ID No:60 and said proteorhodopsin protein is Sequence ID No:61.
- 15 35. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALE6, said proteorhodopsin gene is Sequence ID No:62 and said proteorhodopsin protein is Sequence ID No:63.
- 20 36. The proteorhodopsin gene of claim 2, wherein said genomic fragment is retrieved from a clone PALE7, said proteorhodopsin gene is Sequence ID No:64 and said proteorhodopsin protein is Sequence ID No:65.
- 25 37. The proteorhodopsin gene of claim 1, wherein said proteorhodopsin gene is amplified from a genomic fragment by polymerase chain reaction.
38. The proteorhodopsin gene of claim 37, wherein said polymerase chain reaction is performed by primers with Sequence ID No:2 and Sequence ID No:3.

39. The proteorhodopsin gene of claim 1, wherein said proteorhodopsin gene is derived from a marine environment and placed in an expression vector for producing said proteorhodopsin protein in a host.

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40. The proteorhodopsin gene of claim 39, wherein said host is an artificial membrane system.

41. The proteorhodopsin gene of claim 39, wherein said host is a bacterium.

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42. The proteorhodopsin gene of claim 41, wherein said host is a cell membrane preparation of said bacterium.

43. The proteorhodopsin gene of claim 39, wherein said host is an eukaryote.

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44. The proteorhodopsin gene of claim 43, wherein said host is a cell membrane preparation of said eukaryote.

45. A method of retrieving a proteorhodopsin gene, comprising the steps of:

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- (a) providing a sample of naturally occurring bacteria;
- (b) extracting a genomic fragment of said sample of naturally occurring bacteria;
- and
- (c) amplifying said proteorhodopsin gene from said genomic fragment using polymerase chain reaction.

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46. The method of claim 45, further comprising the step of creating an expression vector containing said proteorhodopsin gene.
47. The method of claim 45, wherein said naturally occurring bacteria are marine proteobacteria.
48. The method of claim 45, wherein said naturally occurring bacteria are SAR86 bacteria.
49. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.
50. The method of claim 49, said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.
51. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC31A8, and wherein said amplified proteorhodopsin gene from said clone BAC31A8 is Sequence ID No:4 and encodes a proteorhodopsin protein according to Sequence ID No:5.
52. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC40E8, and wherein said amplified proteorhodopsin gene from said clone BAC40E8 is Sequence ID No:8 and encodes a proteorhodopsin protein according to Sequence ID No:9.

53. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC41B4, and wherein said amplified proteorhodopsin gene from said clone BAC41B4 is Sequence ID No:10 and encodes a proteorhodopsin protein according to Sequence ID No:11.

54. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone BAC64A5, and wherein said amplified proteorhodopsin gene from said clone BAC64A5 is Sequence ID No:12 and encodes a proteorhodopsin protein according to Sequence ID No:13.

55. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT0m1, and wherein said amplified proteorhodopsin gene from said clone HOT0m1 is Sequence ID No:14 and encodes a proteorhodopsin protein according to Sequence ID No:15.

56. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m1, and wherein said amplified proteorhodopsin gene from said clone HOT75m1 is Sequence ID No:16 and encodes a proteorhodopsin protein according to Sequence ID No:17.

57. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m3, and wherein said amplified proteorhodopsin

gene from said clone HOT75m3 is Sequence ID No:18 and encodes a proteorhodopsin protein according to Sequence ID No:19.

58. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m4, and wherein said amplified proteorhodopsin gene from said clone HOT75m4 is Sequence ID No:20 and encodes a proteorhodopsin protein according to Sequence ID No:21.

59. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone HOT75m8, and wherein said amplified proteorhodopsin gene from said clone HOT75m8 is Sequence ID No:22 and encodes a proteorhodopsin protein according to Sequence ID No:23.

60. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB0m1, and wherein said amplified proteorhodopsin gene from said clone MB0m1 is Sequence ID No:24 and encodes a proteorhodopsin protein according to Sequence ID No:25.

61. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB0m2, and wherein said amplified proteorhodopsin gene from said clone MB0m2 is Sequence ID No:26 and encodes a proteorhodopsin protein according to Sequence ID No:27.

62. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB20m2, and wherein said amplified proteorhodopsin gene from said clone MB20m2 is Sequence ID No:28 and encodes a proteorhodopsin protein according to Sequence ID No:29.

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63. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB20m5, and wherein said amplified proteorhodopsin gene from said clone MB20m5 is Sequence ID No:30 and encodes a proteorhodopsin protein according to Sequence ID No:31.

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64. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB20m12, and wherein said amplified proteorhodopsin gene from said clone MB20m12 is Sequence ID No:32 and encodes a proteorhodopsin protein according to Sequence ID No:33.

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65. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB40m1, and wherein said amplified proteorhodopsin gene from said clone MB40m1 is Sequence ID No:34 and encodes a proteorhodopsin protein according to Sequence ID No:35.

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66. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB40m5, and wherein said amplified proteorhodopsin gene from said clone MB40m5 is Sequence ID No:36 and encodes a proteorhodopsin protein according to Sequence ID No:37.

67. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB40m12, and wherein said amplified proteorhodopsin gene from said clone MB40m12 is Sequence ID No:38 and encodes a proteorhodopsin protein according to Sequence ID No:39.

68. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m5, and wherein said amplified proteorhodopsin gene from said clone MB100m5 is Sequence ID No:40 and encodes a proteorhodopsin protein according to Sequence ID No:41.

69. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m7, and wherein said amplified proteorhodopsin gene from said clone MB100m7 is Sequence ID No:42 and encodes a proteorhodopsin protein according to Sequence ID No:43.

70. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m9, and wherein said amplified proteorhodopsin gene from said clone MB100m9 is Sequence ID No:44 and encodes a proteorhodopsin protein according to Sequence ID No:45.

71. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone MB100m10, and wherein said amplified proteorhodopsin

gene from said clone MB100m10 is Sequence ID No:46 and encodes a proteorhodopsin protein according to Sequence ID No:47.

72. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB1, and wherein said amplified proteorhodopsin gene from said clone PALB1 is Sequence ID No:48 and encodes a proteorhodopsin protein according to Sequence ID No:49.

73. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB2, and wherein said amplified proteorhodopsin gene from said clone PALB2 is Sequence ID No:50 and encodes a proteorhodopsin protein according to Sequence ID No:51.

74. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB5, and wherein said amplified proteorhodopsin gene from said clone PALB5 is Sequence ID No:52 and encodes a proteorhodopsin protein according to Sequence ID No:53.

75. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB7, and wherein said amplified proteorhodopsin gene from said clone PALB7 is Sequence ID No:54 and encodes a proteorhodopsin protein according to Sequence ID No:55.

76. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB6, and wherein said amplified proteorhodopsin gene from said clone PALB6 is Sequence ID No:56 and encodes a proteorhodopsin protein according to Sequence ID No:57.

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77. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALB8, and wherein said amplified proteorhodopsin gene from said clone PALB8 is Sequence ID No:58 and encodes a proteorhodopsin protein according to Sequence ID No:59.

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78. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALE1, and wherein said amplified proteorhodopsin gene from said clone PALE1 is Sequence ID No:60 and encodes a proteorhodopsin protein according to Sequence ID No:61.

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79. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALE6, and wherein said amplified proteorhodopsin gene from said clone PALE6 is Sequence ID No:62 and encodes a proteorhodopsin protein according to Sequence ID No:63.

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80. The method of claim 45, wherein said naturally occurring bacterial genomic fragment is in a clone PALE7, and wherein said amplified proteorhodopsin gene from said clone PALE7 is Sequence ID No:64 and encodes a proteorhodopsin protein according to Sequence ID No:65.

81. The method of claim 45, wherein said polymerase chain reaction is performed by primers with Sequence ID No:2 and Sequence ID No:3.

5 82. The method of claim 45, further comprising the step of providing a host.

83. The method of claim 82, wherein said host is an artificial membrane system.

84. The method of claim 82, wherein said host is a bacterium.

10 85. The method of claim 84, wherein said host is a cell membrane preparation of said bacterium.

86. The method of claim 82, wherein said host is an eukaryote.

15 87. The method of claim 86, wherein said host is a cell membrane preparation of said eukaryote.

88. A light-driven energy generator, comprising:

- 20 (a) a proteorhodopsin protein;
- (b) a host to correctly fold said proteorhodopsin protein in said host, thereby creating an integrated proteorhodopsin protein; and
- (c) a source of retinal to bind covalently to said integrated proteorhodopsin protein, thereby creating a light absorbing pigment.

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89. The light-driven energy generator of claim 88, wherein said proteorhodopsin protein is encoded by a proteorhodopsin gene retrieved from a genomic fragment of a sample of naturally occurring bacteria.

5 90. The light-driven energy generator of claim 89, wherein said naturally occurring bacteria are marine proteobacteria.

91. The light-driven energy generator of claim 89, wherein said naturally occurring bacteria are SAR86 bacteria.

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92. The light-driven energy generator of claim 89, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.

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93. The light-driven energy generator of claim 92, wherein said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.

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94. The light-driven energy generator of claim 89, wherein said genomic fragment is retrieved from a clone, wherein said clone is a member of the group consisting of BAC31A8, BAC40E8, BAC41B4, BAC64A5, HOT0m1, HOT75m1, HOT75m3, HOT75m4, HOT75m8, MB0m1, MB0m2, MB20m2, MB20m5, MB20m12, MB40m1, MB40m5, MB40m12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6 and PALE7.

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95. The light-driven energy generator of claim 88, wherein said host is an artificial membrane system.

96. The light-driven energy generator of claim 88, wherein said host is a cell membrane obtained from a bacterium.

97. The light-driven energy generator of claim 96, wherein said host is a cell membrane preparation obtained from a bacterium.

98. The light-driven energy generator of claim 88, wherein said host is a cell membrane obtained from an eukaryote.

99. The light-driven energy generator of claim 98, wherein said host is a cell membrane preparation obtained from an eukaryote.

100. The light-driven energy generator of claim 88, further comprising a light source for illuminating said light absorbing pigment, whereby said energy generator converts light into biochemical energy.

101. The light-driven energy generator of claim 100, wherein said light source is a fast-pulsed light source.

102. The light-driven energy generator of claim 101, wherein said fast-pulsed light source comprises a mechanism for delivering intermittent fast-light pulses at predetermined time intervals.

103. The light-driven energy generator of claim 100, wherein said light source is a light source exhibiting different predetermined wavelengths.

104. The light-driven energy generator of claim 88, further comprising a mediator for mediating energy generated by said energy generator into chemical, mechanical or electrical energy.

105. The light-driven energy generator of claim 88, wherein said proteorhodopsin protein is selected to determine an absorption spectra of said light absorbing pigment.

106. A method for making a light-driven energy generator, comprising the steps of:

- (a) providing a proteorhodopsin protein;
- (b) providing a host to correctly fold said proteorhodopsin protein in said host, thereby creating an integrated proteorhodopsin protein; and
- (c) providing a source of retinal to bind covalently to said integrated proteorhodopsin protein, thereby creating a light absorbing pigment.

107. The method of claim 106, wherein said proteorhodopsin protein is encoded by a proteorhodopsin gene retrieved from a genomic fragment of a sample of naturally occurring bacteria.

108. The method of claim 107, wherein said naturally occurring bacteria are marine proteobacteria.

109. The method of claim 107, wherein said naturally occurring bacteria are SAR86 bacteria.

110. The method of claim 107, wherein said naturally occurring bacterial genomic fragment is retrieved from a recombinant DNA library.

111. The method of claim 110, wherein said naturally occurring bacterial genomic fragment is retrieved from a bacterial artificial chromosome library.

112. The method of claim 107, wherein said genomic fragment is retrieved from a clone, wherein said clone is a member of the group consisting of BAC31A8, BAC40E8, BAC41B4, BAC64A5, HOT0m1, HOT75m1, HOT75m3, HOT75m4, HOT75m8, MB0m1, MB0m2, MB20m2, MB20m5, MB20m12, MB40m1, MB40m5, MB40m12, MB100m5, MB100m7, MB100m9, MB100m10, PALB1, PALB2, PALB5, PALB7, PALB6, PALB8, PALE1, PALE6 and PALE7.

113. The method of claim 106, wherein said host is an artificial membrane system.

114. The method of claim 106, wherein said host is a cell membrane obtained from a bacterium.

115. The method of claim 114, wherein said host is a cell membrane preparation obtained from a bacterium.

116. The method of claim 106, wherein said host is a cell membrane obtained from an eukaryote.

117. The method of claim 116, wherein said host is a cell membrane preparation obtained from an eukaryote.

118. The method of claim 106, further comprising the step of providing a light source for illuminating said light absorbing pigment, whereby said energy generator converts light into biochemical energy.

119. The method of claim 118, wherein said light source is a fast-pulsed light source.

120. The method of claim 119, wherein said fast-pulsed light source comprises a mechanism for delivering intermittent fast-light pulses at predetermined time intervals.

121. The method of claim 118, wherein said light source is a light source exhibiting different predetermined wavelengths.

122. The method of claim 106, further comprising the step of providing a mediator for mediating energy generated by said energy generator into chemical, mechanical or electrical energy.

123. The method of claim 106, wherein said proteorhodopsin protein is selected to determine an absorption spectra of said light absorbing pigment.

124. A PCR apparatus for amplifying a proteorhodopsin gene from DNA samples of naturally occurring microbial populations using polymerase chain reaction, comprising oligodeoxynucleotide primers with a Watson-Crick base pair complementarity to 5' and 3' ends of said proteorhodopsin gene.

125. The apparatus of claim 124, wherein said primers are according to Sequence ID No:2 and Sequence ID No:3.

126. A method of designing PCR primers, comprising the steps of:

- (a) determining a DNA sequence of a proteorhodopsin gene; and
- (b) based on said determined DNA sequence in (a), designing oligodeoxynucleotide primers with a Watson-Crick base pair complementarity to said 5' and 3' ends of said proteorhodopsin gene.

127. The method of claim 126, further comprising the step of using said oligodeoxynucleotide primers to amplify said proteorhodopsin gene from DNA samples of naturally occurring microbial populations by polymerase chain reaction.

128. The method of claim 127, further comprising the step of cloning said amplified proteorhodopsin gene into an expression vector.

129. The method of claim 126, wherein said primers are according to Sequence ID No:2 and Sequence ID No:3.